REMARKS

Status of the Claims:

Claims 46-66 are pending in the case.

Rejection of claims 46, 48-51, 54, 55, 58 and 64-66 under 35 U.S.C. §102(b):

Claims 46, 48-51, 54, 55, 58 and 64-66 stand rejected under 35 U.S.C. §102(b) as anticipated by Martino, *et al.* This publication purportedly discloses the same invention, specifically to the extent that the authors report use of ultra rapid cooling of oocytes by dropping microdroplets of an oocyte suspension onto a solid metal grid at liquid nitrogen temperatures. The Action also asserts that the solutions for suspending and rinsing the oocytes is the same as that disclosed by applicants, that the final product retains viability and morphology, and that the oocytes are exposed to the cryopreservation cooling for the same amount of time used by applicants.

Applicants have reviewed the Martino, *et al.* reference recognizing that in order for anticipation to occur, each and every element of the claims must be virtually the same as disclosed in a single reference. Applicants respectfully submit that there are significant differences between any elements described in the Martino, *et al.* reference and applicants' claims.

As nearly as applicants can determine from a study of the Martino, *et al.* reference, oocytes "suspended in EG5.5 were cooled...ultra-rapidly..." then dropped (in one series of experiments) onto a copper grid, which was then blotted from underneath before plunging into liquid nitrogen (page 1061, col 1, last paragraph). There is no indication that oocytes are first equilibrated in an equilibration medium and then rinsed in a vitrification solution prior to vitrification. This alone is failure of Martino, *et al.* to teach each element of Applicants' invention.

Additionally, applicants find nothing in the publication to suggest the steps described in the present application prior to cryopreservation. The media used by Martino, *et al.* for collection and *in vitro* maturation (see page 1060 under Material and Methods) appears to be standard

culture media. A brief reference to exposure to "cryoprotectant" does not equate with applicants' equilibration and vitrification solutions.

The Martino, *et al.* paper therefore does not anticipate applicants' invention because it does not teach or even suggest each and every element of the invention.

Rejection under 35 U.S.C. §103:

Claims 46-51, 54-62 and 64-66 have been rejected under 35 §103(a) as unpatentable over Martino, *et al.* and Papis, *et al.* The Martino, *et al.* reference purportedly discloses use of metal grids dipped into liquid nitrogen to rapidly cool oocytes suspended in vitrification solution with ethylene glycol as an intracellular cryoprotectant. Applicants see no such disclosure in the Martino, *et al.* publication. They note a reference to a mixture of ethylene glycol and sucrose which was used for CPA solutions, but there is no reference to the use of a vitrification solution *per se* nor to an equilibration solution, much less the steps for how and when these solutions should be used.

Papis, *et al.* disclose an ethylene glycol pretreatment of oocytes prior to vitrification. A pre-equilibration is performed in ethylene glycol in TCM 199 medium supplemented with fetal calf serum at about 2-5 degrees below body temperature for about 12-15 min. This is different from the temperature of the equilibration solution used by Applicants, which is at or above body temperature. Relatively small temperature differences for biological materials may be significant. Additionally, Papis, *et al.* use a significantly different vitrification medium consisting of ethylene glycol and sucrose. Applicants' vitrification medium contains four components: namely, a cryoprotectant such as ethylene glycol; a sugar, a macromolecule and a surfactant.

Combining the Martino, *et al.* reference with Papis, *et al.*, one of skill in the art would be likely to use the same solutions described by Papis, *et al.* when rapidly vitrifying the oocytes. There is no teaching in the combined references to use the equilibration and vitrification solutions used by

applicants, nor is there any motivation to do so. Applicants' data, particularly in Tables 1 and 4 of the application show the improved results from practice of Applicants' method.

The benefit of Applicants' method is, admittedly, in the results; Papis, *et al.* in Table 1 report 8 hatched blastocytes (15%) after 10 days compared with 21% for a control; in contrast, Applicants report 20% hatched blastocytes after 9 days compared with 22% for a control. Applicants believe that their results represent a significant improvement in oocyte cryopreservation.

Rejection of Claims 46-66 under 35 U.S.C. §103:

Claims 46-66 have been rejected as unpatentable over Martino, *et al.* in view of Papis, *et al.*, and further in view of Arav, *et al.;* Saha, *et al.*; and Liu, *et al.* The Papis *et al.* reference is asserted to demonstrate that optimization protocols for cryopreservation require multiple steps including the use of plastic straws rather than metals or metal grids. Martino, *et al.* is cited as showing that metal grids could be used in place of straws in optimizing viability of oocytes or embryos. Arav, *et al.* is cited as disclosing the use of trehalose as a cryoprotectant that is superior to sucrose. Saha, *et al.* is cited as disclosing use of ethylene glycol, trehalose and polyvinylpyrollidone for vitrification and oocyte preservation solutions. Liu, *et al.* is relied upon to show that culturing fertilized oocytes in KSOM medium with cumulus cells is known in the art.

Applicants recognize that the art clearly illustrates the problems in developing methods of cryopreservation that allow long term storage of oocytes while maintaining morphology and viability. In fact, all the references indicate a struggle to achieve this goal. Yet the combination of any of the cited references with Martino, *et al.* leads to more, rather than less, confusion as to what steps to use that best lead to the desired result. Arav, *et al.* state that, with regard to the many attempts to develop effective cryoprotectant solutions in view of the many different experiments to use cryoprotectant resulting in toxicity and osmotic injury upon thawing (col 1, first paragraph, under Discussion), "We suggest that the use of this glycoprotein together with

the vitrification solution proposed here could be a great challenge for reaching a successful cryopreservation of immature bovine oocytes." (page 357, col 2, last paragraph in Discussion). Applicants believe that this is strong evidence of the undue experimentation contemplated by Arav, *et al.* that will be required to improve cryopreservation methods. The Arav, *et al.* provides further support that there is no motivation to combine any of the teachings of the secondary references with Martino, *et al.*

In summary, Applicants submit that their method of oocyte cryopreservation is a significant improvement in the art, that their method differs from the methods suggested or used by others, and that Applicants' results are not an obvious combination of selection of the many different steps proposed for oocyte cryopreservation found in the cited references.

This paper is intended to be a complete response to the examiner's action. Should the examiner have any questions or suggestions, the undersigned respectfully requests a telephone conference at 203.353.6848.

Respectfully Submitted,

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